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European Research Office (9851 DU),
U.S. Department of the Army,
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Studies on the toxins of
Pseudomonas cocovenenans.

Name of Contractor:

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SUMMARY.

I. Bongkreki acid.

Fatal food poisonings have repeatedly occurred amongst the natives of the densely populated parts of Mid-Java in Indonesia. These were caused by the eating of coconut-products ("bongkreki") that had been inoculated with moulds (*Rhizopus oryzae*). A study of the background of these poisonings was made round about 1930. An extensive investigation brought to the light the fact that sometimes a bacterium developed instead of the mould with which the defatted coconut was inoculated and this secreted a very active poison. This "bongkreki bacterium" was identified as belonging to the genus *Pseudomonas* and it got the name "*Pseudomonas cocovenenans*". The toxic compound, bongkreki acid, was isolated from cultures of this microorganism on moist, defatted copra. This isolation was worked out by using extraction procedures followed by thin layer chromatography or liquid-liquid chromatography on Sephadex. The concentration of bongkreki-acid at the different phases of isolation was determined by measuring the UV-absorption or the antibiotic activity against *Cladosporium cucumerinum*.

Bongkreki acid appears to be a highly unsaturated fatty acid. The elucidation of the structure has yet to be done.

Bongkreki acid poisonings are characterized by a severe hyperglycaemia soon followed by hypoglycaemia. Though some work on the pharmacological action of bongkreki acid/this must now be considered as out of date. For that reason several samples of bongkreki acid have been forwarded to the Army Chemical Center to make possible a thorough pharmacological testing.

/ has been done

SUMMARY.

II. Compounds containing an unsymmetrical triazinering.

- a. After initial experiments starting from amino-acetonitrile and α -aminopropionitril that were not successful 3,5-dihydro-1,2,4-triazine-6-carboxylic acid could be synthesized by a Curtius-degradation of 3,5-dihydroxy-1,2,4-triazine-6-carboxylic acid. A number of interesting, previous unknown compounds were obtained as intermediates.
- b. The synthesis of pyrazolo-pyrimidine has also been described.
- c. Substituted and unsubstituted hydrazino-nitropyridines could be prepared. The reduction of these compounds, only possible if the hydrazino group is protected by an acylgroup, has been accomplished. These compounds will be used as starting materials for the synthesis of pyridino-as-triazines. Uptill now we have been able to obtain 1,3-dimethyl-1,4-dihydro-pyrido (3,2-e)-1,2,4-triazine hydrochloride by ring closure.

Detailed descriptions of the preparation of all the previous unknown compounds are given.

BONGKREKIC ACID.

In the past, cases of fatal food poisoning, due to the consumption of coconut products (bongkretek), inoculated with moulds (*Rhizopus oryzae*), occurred amongst the natives of Banjoemas, a district of Mid-Java. As early as in the beginning of the thirties Mertens and van Veen studied the cause of these poisonings. They brought to light, that the toxine involved, which they called bongkretekic acid, is produced by a bacterium now classified as *Pseudomonas cocovenenans* on partially defatted coconut-products.

Though some work on the pharmacological activity of bongkretekic acid has been done, this must be considered out of date.

Bongkretekic acid poisonings are characterized by a severe hyperglycaemia soon followed by hypoglycaemia. Administration of glucose did not prevent the death of rats, so the hypoglycaemia itself is apparently not responsible for the lethal effect. The cumulative effect of bongkretekic acid and the sudden onset of a shock-condition are noteworthy. No definite damage to the Langerhaus-islets could be established. The toxicity of bongkretekic acid is very general (human beings, monkeys, rats, rabbits, pigeons) and the substance also shows powerful antibiotic properties (yeasts, bacteria, moulds).

Lyophilized samples of crude bongkretekic acid preparations were forwarded to the Army Chemical Center for a repetition of pharmacological experiments. We have been informed about some results of preliminary work.

Chemically bongkretekic acid is a highly unsaturated fatty acid of still unknown structure. The isolation and some work on the chemistry of this product has been described by Nugteren and Berends (*Rec.trav.chim.* 76 (1957) 13). Several laboratories wanted to continue this work and therefore we decided to stop it. When, however, after five years no progress had been reported we decided to restart it. So we resumed the work on bongkretekic acid in November 1962.

The first thing we wanted to do was to improve the cultivation of the microorganism on a more extensive scale. At present we are able to prepare a few grams of crude bongkretekic acid weekly.

The bongkreki acid is produced by Pseudomonas cocovenenans on partially defatted coconut products. Though a submerged culture would make a larger production much easier, it appears that no bongkreki acid is formed, if the partially defatted coconut-product is omitted. The strong antibiotic properties of bongkreki acid and the fact that toxoflavine is formed as well make it unnecessary to work under severe sterile conditions.

From the isolation scheme (see experimental part), leading to the crude bongkreki acid, it is clear that fatty acids are not excluded. The preparations described by van Veen and Hertens must have been very impure. Nugteren in this laboratory purified the crude bongkreki acid by using a chromatopile but at present we are doubting whether he really obtained a pure compound.

It appears that the amount of the impurities in the crude bongkreki acid preparations is dependent on the time of incubation. After a short cultivation the mixture contains only a few impurities in rather small concentrations. The longer the incubation time the greater the number of the impurities and their concentrations.

Methods of determination of bongkreki acid.

At the various stages of the isolation the concentration of bongkreki acid can most easily be determined spectrophotometrically (bongkreki acid shows a very intensive absorption at 268 and 239 mμ). However, there are compounds in the crude mixture which show an absorption in the same region. Consequently no exact figure about the concentration can be deduced from the extinction.

A very sensitive method for the determination is based on the high antibiotic activity towards Cladosporium cucumerinum.

Though this microbiological test is qualitatively excellent it is nevertheless hardly to use for a quantitative determination because there is only a linear relation between concentration and zone of inhibition within a short range.

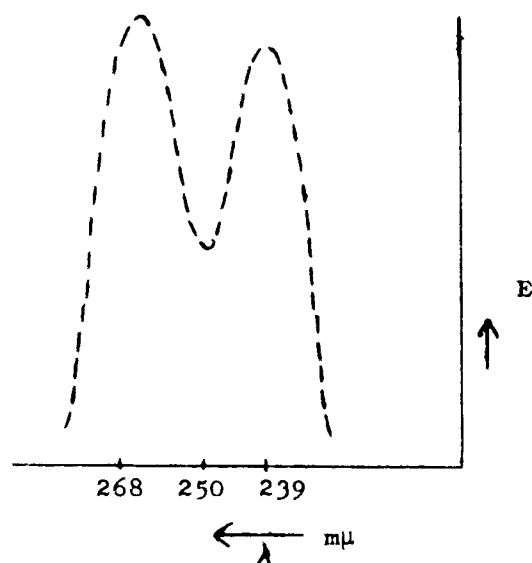
According to our experience the best method for investigating the composition of the mixture makes use of chromatography. Paper-chromatography, however, was not found very successful. Much

better results were obtained by using thin-layer chromatography on silica GF₂₅₄. A mixture of chloroform, methanol and acetic acid (94:5:1) proved to be useful as eluent. The unsaturated compounds are visible in U.V. light on the chromatoplate as dark spots. All acids may be demonstrated by spraying with a bromocresolgreen solution in ethanol and exposing the plate to ammonia (the acids form yellow spots on a blue background). Unsaturated compounds can also be detected by spraying with a saturated solution of SbCl₃ in CHCl₃ or by spraying with alkaline-KMnO₄.

Only one of the spots present appeared to possess antibiotic properties.

Purification,

- A. It has been found very difficult to transfer the thin-layer chromatography separations to a preparative scale. However, after a lot of experiments we have nevertheless succeeded. In the first place by increasing the thickness of the adsorbent-layer on the plate the capacity of the chromatoplate could be increased. It appeared possible to prepare plates covered with a layer of 1 mm silica. After development of the chromatoplate the bongkreikic acid band was scratched from the plate and the resulting powder was extracted with methanol. The methanolic extract had to be filtered over an asbestos filter (Seitz) to remove small particles of silica and gypsum. After evaporation of the methanol in vacuo bongkreikic acid was left behind as a colourless oil, which solidified to an amorphous white powder, that can be redissolved in methanol. The bongkreikic acid prepared in this way seemed to be highly pure for it only showed one sharp spot on the chromatogram. In connection with the results from counter-current distribution studies we may conclude that this spot represents only one compound. The spectrum of this material shows two maxima at 239 mμ and 268 mμ and a minimum at 250 mμ.



Ratio of the maxima:

$$\frac{E_{239}}{E_{268}} = 0,98.$$

Extinction 1 mg B.A./ml \approx 59.

B. Another way of separating bongkreki acid from the accompanying compounds was found in using a horizontal column of silica in a cellophane tube (H. Dahn and H. Fuchs - *Helv.Chim.Acta* 45 (1962) 261).

The mixture was brought on that side of the column from which the solvent is sucked in by capillary force. The silicagel used contained fluorescent powder in order to be able to locate the compounds.

After development the column was cut in slices, which were eluted and tested. About 15 mg. bongkreki acid per column could be obtained in this way. This method, however, is more time-consuming than the thin-layer chromatography described above.

C. From the isolation scheme it may be expected that fatty acids will be present in crude preparations of bongkreki acid. Attempts were made to remove unbranched fatty acids by the formation of inclusion compounds in urea. It was found that some of the fatty acids could be removed for a large part in this way; but most of the other accompanying fatty acids did not form clathrate compounds.

D. The purification of bongkreki acid was also tried by counter-current distribution. So far no solvents-mixture has been found

that gave satisfactory results in the countercurrent distribution of crude preparations.

This work on the counter-current distribution, however, has led us to experiments on a liquid-liquid chromatography on a Sephadex column. The stationary phase in this kind of chromatography is water. Organic solvents may be used as eluents. Preliminary experiments look promising.

EXPERIMENTAL PART.

Pseudomonas cocovenenans.

The microorganism, producing bongkreikic acid (Ps.cocovenenans) was present in the laboratory as freeze-dried cultures. These preparations were made by Nugteren in 1957. After incubation for four days at 25° C in meat bouillon-2% glycerine various colonies of bongkreik acid producing bacteria were isolated via 1% glycerine-1% pepton, 2% agar plates. By transferring the bacteria every 14 days into 1% pepton - 2% glycerine - 2% agar - $\frac{1}{2}$ % NaCl tubes and incubating them at 30° C cultures were obtained that showed a satisfactory constant production of bongkreikic acid.

Another culture, which had been used for the production of toxoflavine, gave nearly the same yield of bongkreikic acid.

Preparation of bongkreikic acid.

2,5 kg. commercial shredded copra was soaked for several hours in water at 80° C. When the temperature had been dropped to about 40° C the paste was squeezed out in a cloth. This treatment was repeated. The wet product ("ampas") is divided into 12 dishes (diameter 21 cm., height 10 cm.) making a layer of 1 to 2 cm. deep.

After sterilisation at 110° C for 1 hour the dishes were inoculated with about 25 ml. each of a culture of Ps.cocovenenans in 1% peptonwater - 2% glycerine, which has been incubated for 24 hours at 30° C. The covered inoculated dishes were kept at 30° C and a relative humidity of 60%. The covers were supported by rubber

rings, which had been attached on the rim of the dish. This construction is necessary to obtain a good aeration and to avoid the ampas might become too wet. After 24 hrs. incubation the ampas was yellow coloured at the places of inoculation.

The covers ought to be dried and the contents of the dishes should be aired and stirred daily. After 2 or 3 days the ampas became pale brown and after 4 days the covers were removed and the material was allowed to dry out.

Isolation of bongkreki acid.

The dry ampas (1 - 1,5 kg.) was twice extracted with petroleum ether (b.p. 60-80° C). This was done by digesting the ampas with petroleum ether at roomtemperature for 24 hrs. The petroleum ether extract was extracted with 50 ml. lots of 8% sodiumbicarbonate solution.

The combined bicarbonate extracts were acidified with 2N H₂SO₄ to pH=3 and extracted with 250 ml of peroxide-free ether. The ethereal solution was washed twice with 100 ml. of water and then extracted with 25 ml. portions of 8% sodiumbicarbonate solution, untill the extracts did not show a large absorption at 263 mμ anymore. In this way about 2 à 3 g. of crude bongkreki acid, could be obtained from 1 à 1½ kg. ampas. Usually the bongkreki acid was transferred to an ammonia solution. This was done by acidifying the solution with 2N H₂SO₄ to pH=3 and extracting with peroxide-free ether. The ethereal solution was washed twice with water and extracted with 2N ammonia.

A solution of about 50 mg/ml. bongkreki acid (dry weight 130 mg/ml.) could be obtained in this way.

Determination of bongkreki acid.

a. By ultraviolet absorption.

See figure B under C.

E₂₃₉, E₂₅₀ and E₂₆₈ were measured. Because we don't know $\epsilon_{B.A.}$ and the ϵ -value of the impurities, we can only conclude from the spectrum that bongkreki acid is present.

b. By antibiotic activity.

Bongkrekie acid is very active towards Cladosporium cucumerinum.

For making a testplate, 10 ml. of culture medium (2% corn-steep liquor; 2% glucose; 3% can sugar melasse; 0,1% $MgSO_4$; 2% agar in tap water; pH=6) were melted up and after cooling to $45^\circ C$ mixed with 2 ml. of a suspension of spores of Cladosporium cucumerinum, after which the still liquid medium was poured out directly into a large Petri-dish (diam. 8 cm.). On the cooled agar plate can be placed: a piece of poisonous copra or a drop of a solution, containing bongkrekie acid. After 24 hrs. incubation at $25^\circ C$ clear rings were visible around the places where bongkrekie acid was present.

Cladosporium cucumerinum was transferred every 4 weeks into moutagar tubes and was incubated at $25^\circ C$.

c. Thin-layer chromatography.

The chromatoplates were made in the usual manner. Adsorbens silica GF₂₅₄. Thickness of the layer: 0,25 mm. Eluent was a mixture of chloroform, methanol and acetic acid (94-5-1). Indicator solution: 0,04% bromocresolgreen in ethanol. The unsaturated compounds may be detected under U.V.-light as dark spots or by a spray of alkaline KNO_3 (0,5% in 2N soda-solution), after spraying with the indicator solution. (Green spots on a purple brown background).

After development of the chromatogram it looks as shown in fig. A.

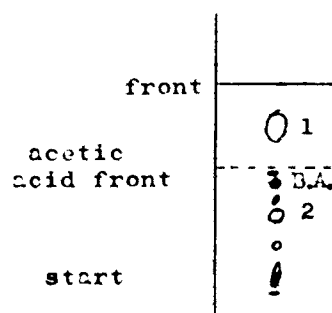
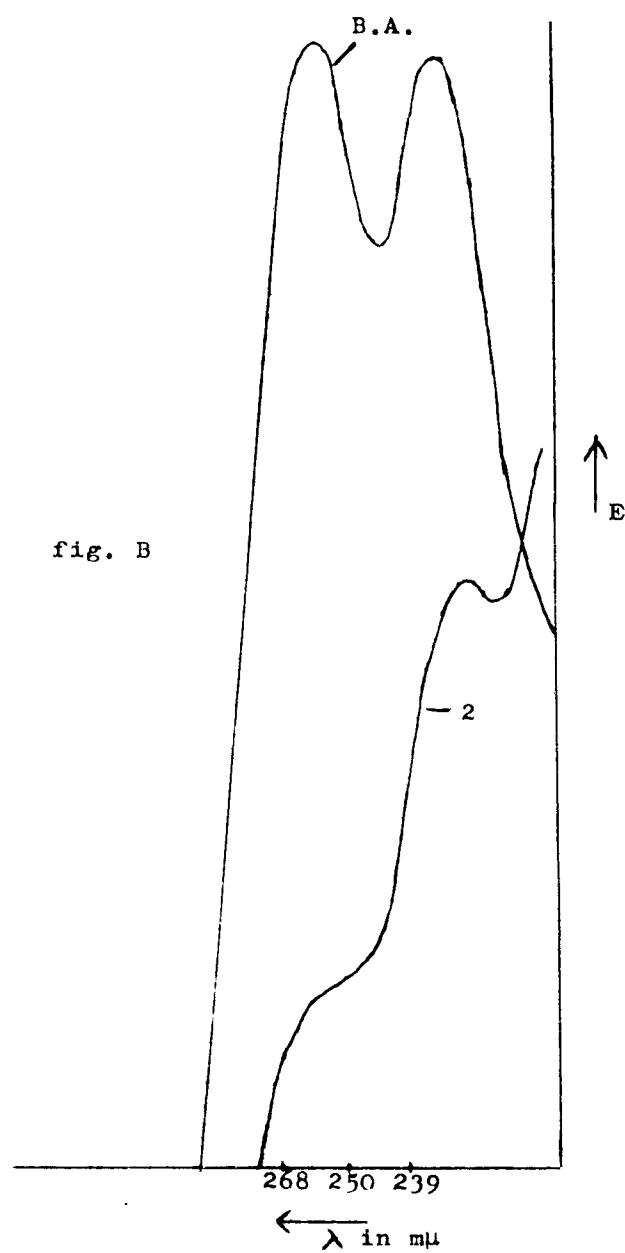


fig. A

1 = saturated fatty acids.

B.A. = bongkrekie acid.

2 = unsaturated compound, which is the main unsaturated impurity.



Purification.

A. Thin-layer chromatography.

Preparation of the plates: a slurry of silica GF₂₅₄ was made (2 water : 1 silica) and the glass plate (20x20 cm.) was coated with a layer of 1 mm. thickness. The plate was allowed to dry at roomtemperature. After that the plate was slowly (3 hrs!) warmed up in an oven to 110° C. and cooled to roomtemperature (slowly again 3 hrs!).

1 ml. (130 mg. dry weight) of the crude bongkrekie solution in 2N ammonia was acidified with 2N H₂SO₄ and extracted with peroxide-free ether. The ethereal solution was washed twice with water and dried over Na₂SO₄. Then the ether was evaporated and the rested oil solved in methanol.

The methanolic solution was sucked in the silica on the plate to a height of 2 cm. The plate was allowed to dry and the rest of the solution was sucked in the silica (1).



The absorbed band was pressed together to a small band by using a solution of methanol-1% acetic acid (2).

The chromatoplate may be developed by a mixture of chloroform-methanol, acetic acid (94-5-1). After development the plate was allowed to dry and examined under U.V. light. The separation was not yet complete. So it was developed again, which resulted in a good separation (3). The bongkrekie acid band was removed from the plate and the silica-powder extracted with methanol. The methanolic extract was filtered over an asbestos filter (Seitz) and evaporated to dryness. Residu: 25 mg. of chromatographically pure bongkrekie acid.

B. Horizontal column.

H. Dahn and H. Fuchs, Helv.Chim.Acta 45 (1962) 261. The

column is made as described by Dahn using silica HF₂₅₄ as sorbent in a cellophane tube with a diameter of 1,8 cm.

60 mg. of the crude mixture was adsorbed at 500 mg. of silica from an ethereal solution. The ether was evaporated in vacuo. When the solvent-front had run 20 cm. from the start, the tube was cut off, so that the solvent could evaporate. After 20 hours two bands had been separated (controlled by U.V. light). They were cut out and tested. 15 mg. of chromatographically pure bongkrekic acid could be obtained.

C. Urea complexes.

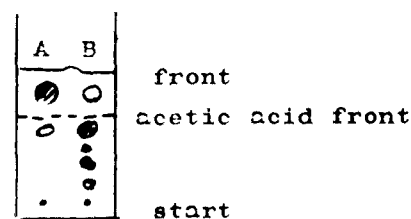
10 ml. of the crude solution in 2N ammonia were acidified by 2N H₂SO₄ and extracted with peroxide-free ether. The ethereal solution was dried over Na₂SO₄ and after filtration the ether was evaporated.

The residu (1,21 g. oil) was dissolved in 24 ml. methanol-15% ureum.

After standing overnight a few crystals had been formed. After 4 hours at 2° C. 410 mg. of a crystalline precipitate (washed with cold methanol-15% ureum and dried in vacuo) had separated.

The precipitate was treated with water. Small oil droplets could be seen, which were solved in petroleum ether (b.p. 60-80°C). This petroleum ether extract (A) was tested on the chromatoplate (see figure).

One ml. of the methanolic solution was treated with 5 ml. water and extracted with petroleum ether (b.p. 60-80° C). This extract (B) was tested (see figure).



D. Counter-current distribution.

For counter-current distribution experiments a system of 0,5 molar phosphate buffer pH=6,34/di-n-butylether was used.

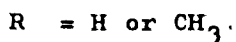
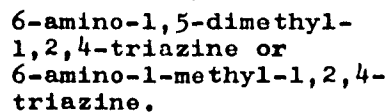
In this system bongkrekic acid showed a K-value ≈ 1 . Some

impurities, however, apparently had the same K-value.

E. Sephadex-column.

A 0,5 molar phosphate buffer pH=6,34 was absorbed by Sephadex G-50. To this buffer solution 0,5 % Na-laurylsulfate had to be added in order to avoid that the column stops running. As eluent we used a gradient of cyclohexane-dibutyl-ether (10-60%).

About 600 mg. (dry weight) of the crude mixture was applied to the column. The larger part of bongkreikic acid was obtained in a chromatographically pure state.

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We have been able to synthesise α -carbobenzoxyamino-propionitrile (II, $R=CH_3$) but all further experiments with this

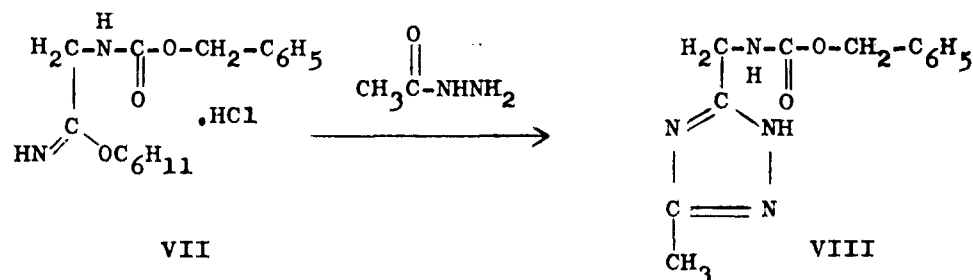
compound have proven fruitless.

In the other series (R=H) we were able to synthesise: carbobenzoxyaminoacetonitrile (II, R=H), carbobenzoxyamino-acetiminocyclohexylether.hydrochloride (III, R=H), carbobenzoxyamino-acetamidrazon.hydrochloride (IV, R=H) and aminoacetamidrazon.dihydrobromide (VI, R=H).

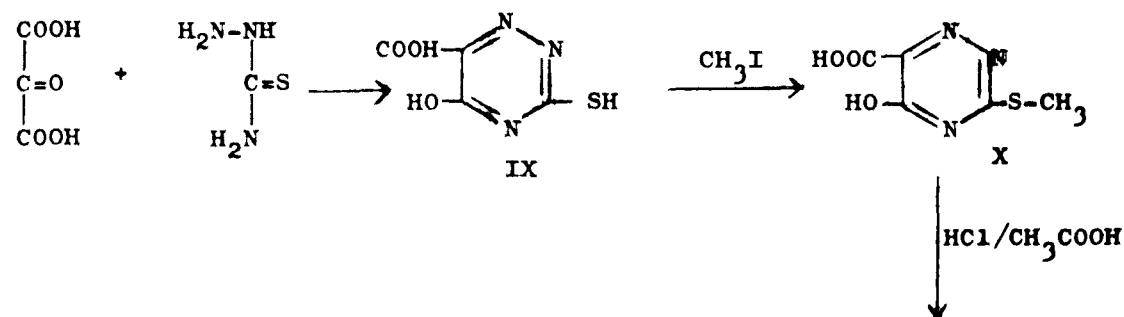
Reaction of iminoether (III, R=H) has to be performed under special conditions since otherwise, instead of amidrazon (IV, R=H) a tetrazine (V) will be formed.

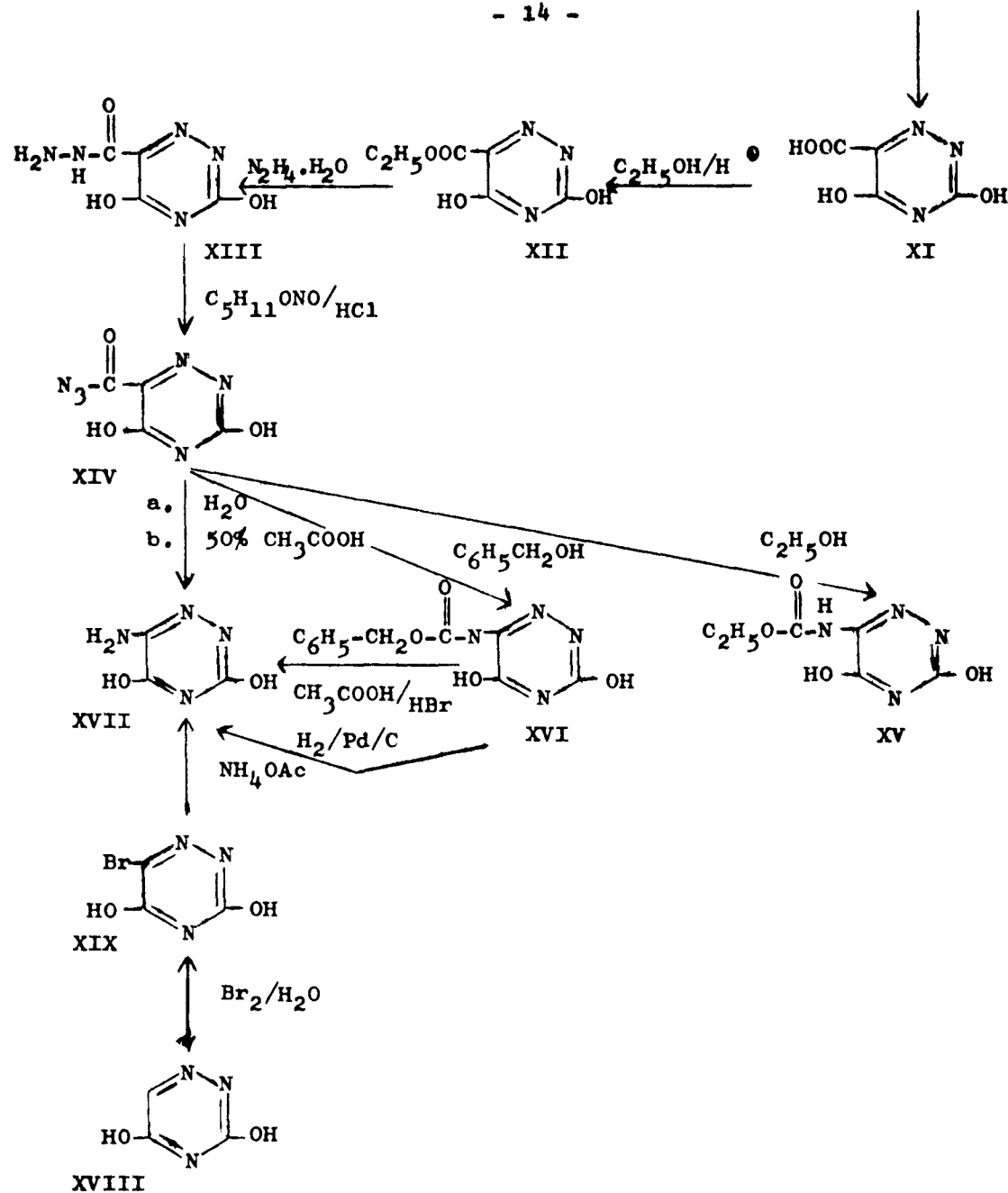
Ringclosure could not be performed; the amidrazon is decomposed too easily. Therefore we did not continue this work.

According to J.Chem.Soc. (1962) the condensation of an iminoether with an acylhydrazide leads to an unsymmetrical triazole. We tried the condensation of carbobenzoxyamino-acetiminocyclohexylether.hydrochloride (VII) with acetylhydrazide in the presence of an equivalent amount of sodium hydroxide. A crystalline compound was obtained, which appeared to be 3-methyl-5-carbobenzoxyamino-1-methyl-1,2,4-triazole.monohydrate (VIII):



b. Though 6-amino-3,5-dihydroxy-1,2,4-triazine was already synthesised by P.K. Chang (J.Org.Chem. 26 (1961) 1118-20), we decided to try to synthesise it by another way:





Though some of the compounds in this scheme are known we nevertheless found it interesting to undertake this synthesis by using a Curtius degradation.

First we have tried to obtain 3,5-dihydroxy-1,2,4-triazine-6-carboxylic acid (XI) by oxidation of the 3-mercaptogroup of compound (IX) with potassium permanganate followed by acidification with hydrochloric acid. According to Falco and coworkers (J.Am.Chem.Soc. 78 (1956) 1938) a sulphonic acid is formed as intermediate which is subsequently hydrolysed by hydrochloric acid.

We obtained, however, a potassium sulphonate from which the potassiumion is very difficult to remove. Therefore we methylated the mercaptogroup with methyl iodide giving 3-methylmercapto-5-hydroxy-1,2,4-triazine-6-carboxylic acid (X). The methylmercapto-group could easily be hydrolysed with a mixture of hydrochloric acid and glacial acetic acid (R.B. Barlow, J.Am.Chem.Soc. 78 (1956) 1258). In this way we obtained 3,5-dihydroxy-1,2,4-triazine-6-carboxylic acid (XI).

Esterification was accomplished by refluxing the compound (XI) in absolute alcohol in the presence of 3% sulphuric acid. The ester: 6-carboethoxy-3,5-dihydroxy-1,2,4-triazine (XII) could be isolated in a yield of 70%.

Reaction of the ester with hydrazinehydrate in absolute alcohol gave the hydrazine salt of 3,5-dihydroxy-1,2,4-triazine-6-carboxyhydrazide (XIII) in an almost quantitative yield.

Formation of 3,5-dihydroxy-1,2,4-triazine-6-carboxyazide (XIV) with sodiumnitrite and hydrochloric acid in water in analogy to Berends and coworkers (Recueil 80 (1961) 391) gave a yield of 60%. Due to the high solubility of the azide in water one had, to obtain this yield, to work in a very small volume, and the results are not reproducible. Reaction with isoamylnitrite and hydrochloric acid in absolute alcohol was more successful (Jensen, Howland, J.Am.Chem. Soc. 48 (1926) 1988). In this way yields of 70-75% were obtained. Though we have no analytical data on this compound, the explosive character at about 150° C is in agreement with an azide.

Reaction of 3,5-dihydroxy-6-carboxyazide-1,2,4-triazine (XIV) with ethanol or benzylalcohol gave respectively 3,5-dihydroxy-6-carboethoxyamino-1,2,4-triazine (XV) and 3,5-dihydroxy-6-carbobenzoylamino-1,2,4-triazine (XVI).

These urethanes have to be transferred into the amino-compound. Since the ethylurethane was very stable to chemical reactions, only the benzylurethane was converted into 6-amino-3,5-dihydroxy-1,2,4-triazine (XVII). Reaction of the benzylurethane with a 33% solution of hydrobromic acid in glacial acetic acid or catalytic hydrogenation with palladium on charcoal gave this aminocompound.

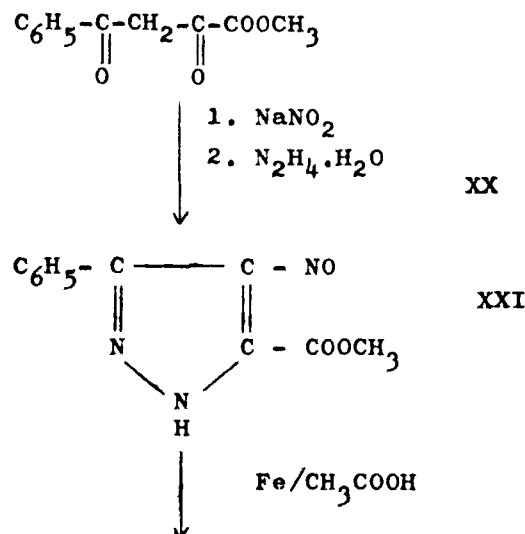
The amino-as-triazine has also been formed in analogy to the work of Bäumlér c.s. (Helv.Chim.Acta 34 (1951) 496) who described a direct method for conversion of an azide into an aminocompound.

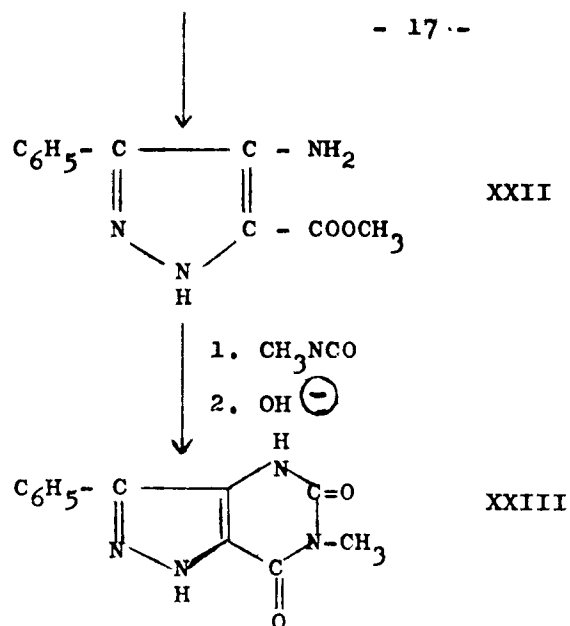
A suspension of azide in 50% acetic acid was gently heated to 100° C in 30 minutes until a clear solution was formed. Upon cooling the aminocompound was obtained. Direct conversion of azide is also possible by heating it in a suspension in water.

The data of the amino compound were in agreement with those found by Chang. She synthesised this compound by bromination of 3,5-dihydroxy-1,2,4-triazine(6-azauracil XVIII) and substituting the bromine atom by ammonium with ammonium acetate.

The synthesis of 1-methyl-4-phenyl-2,7-dioxo-1,2,3,7-tetrahydro-pyrazolo-(3,4-d)-pyrimidine.

Though we have already reported the successful synthesis of this compound, the experimental data will now be given. The reaction scheme was:



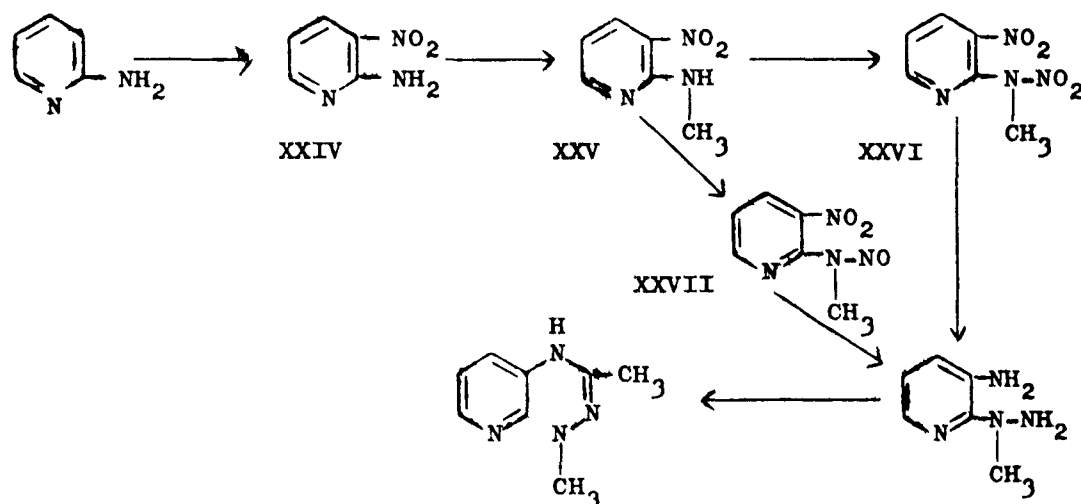


Benzoylpyruvic methylester (XX) was synthesised according to a prescription of M. Freri (Gazz.Chim.Italia 68 (1938) 612-618).

Conversion of this compound, dissolved in glacial acetic acid, with subsequently sodium nitrite and hydrazinehydrate gives 3-phenyl-4-nitroso-5-carbomethoxypyrazole (XXI). Reduction of this compound with iron dust and acetic acid gives 3-phenyl-4-amino-5-carbomethoxypyrazole (XXII).

Ringclosure with methylisocyanate has been done in analogy with Schmidt et al. (Helv.Chim.Acta 42 (1959) 349).

The synthesis of a pyridino-as-triazine has been tried by using the following reaction scheme:

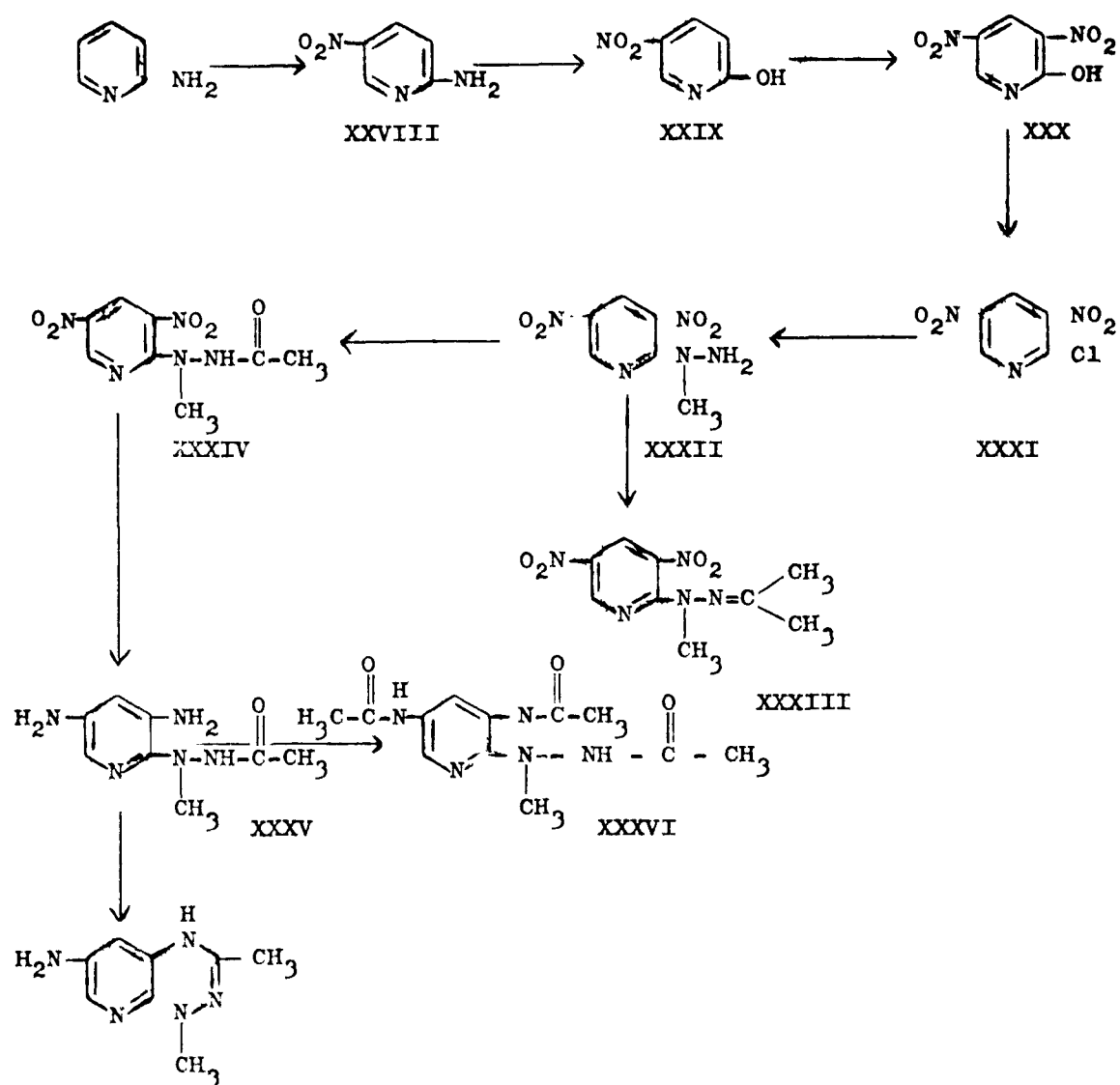


Intermediates XXIV, XXV, XXVI and XXVII have been described by Tschitschibabin and have been synthesised according to his prescriptions.

The reduction of 2-nitrosomethylamino-3-nitropyridine (XXVII) proved to be difficult. Catalytic hydrogenation with palladium on charcoal in several solvents resulted in the 2-methylamino-3-amino-pyridine. The hydrazinogroup is decomposed under these circumstances. Reduction with aluminium amalgam or zink-acetic acid gave the same product.

Even the most gentle reduction; boiling the compound in a suspension of ferrihydroxide under a continuous nitrogen stream (Mager and Berends, Recueil 77 (1959) 5-21) resulted in the same product.

As the reduction of 2-nitrosomethylamino-3-nitro-pyridine was much more difficult than expected, a new series of experiments had been started:



As 2-amino-5-nitropyridine (XXVIII) is the main product in the nitration of 2-amino-pyridine, we decided to use it for the synthesis mentioned above.

The intermediates XXVIII, XXIX, XXX and XXXI have been described in literature and this work has been reproduced.

Adding a cold solution of 2-chloro-3,5-dinitropyridine (XXI) to an excess of methylhydrazine gave the methylhydrazinocompound (XXII) in a yield of 70-75%. The position of the methylgroup was ascertained by the formation of a hydrazone.

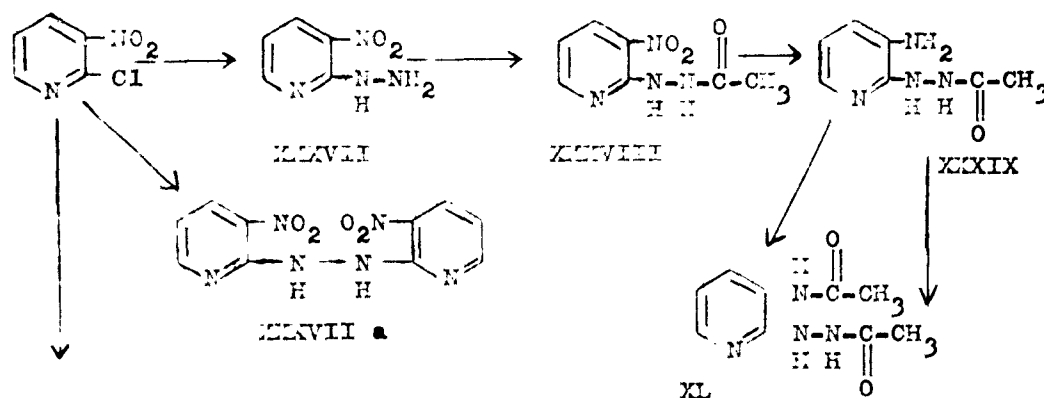
Reaction of the methylhydrazinocompound with acetone and hydrochloric acid gave a solid which appeared to be 2-(1-methyl-2-isopropylidene)hydrazino-3,5-dinitropyridine (XXIII).

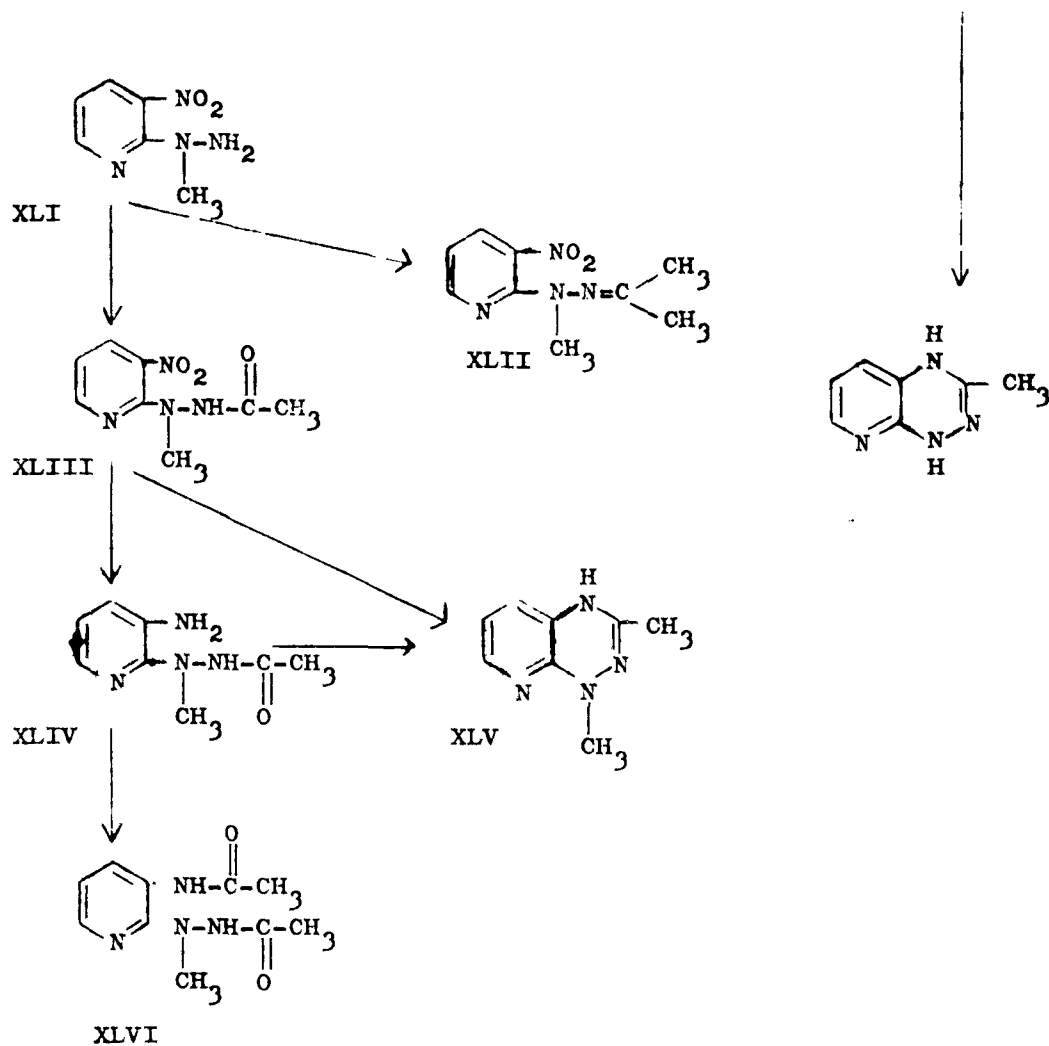
For the next step we dissolved the methylhydrazinocompound at roomtemperature in acetic anhydride. After some time standing at roomtemperature, excess acetic anhydride was removed under diminished pressure, and the solid recrystallised from 50% ethanol. This gave a 2-(1-methyl-2-acetyl)hydrazino-3,5-dinitro-pyridine (XXIV) in an almost quantitative yield.

Reduction of this compound by hydrogenation with palladium on charcoal as catalyst and alcohol as solvent has been successful.

Isolation of the diaminocompound proved to be impossible, as it appeared to be very susceptible to oxygen. However, after removing catalyst and alcohol with exclusion of oxygen, it was possible to obtain the diacetyl derivative, by immediately taking up the residu in acetic anhydride. This gave 2-(2-acetyl-1-methyl)hydrazino-3,5-diacetamido pyridine (XXVI), which was confirmed by analysis.

In the meantime an analogous series of reactions was in progress:





Boiling a solution of 2-chloro-3-nitropyridine in alcohol with an excess hydrazine gave 2-hydrazino-3-nitropyridine (XXXVII) in a yield of 90%. If less than one equivalent hydrazine is used the sym.-di-2,2'-(3-nitropyridyl)-hydrazine (XXXVIIa) is formed.

Dissolving the hydrazinocompound in acetic anhydride and removing excess reagent after some time standing, gave 2-(2-acetyl)hydrazino-3-nitro-pyridine (XXXVIII) in a yield of 90%.

Acetylation is necessary since otherwise the hydrazinogroup is decomposed at reduction.

Hydrogenation of this compound in alcohol with palladium on charcoal gives satisfactory results. The required amount of hydrogen is taken up and the solution is colourless after hydrogenation. Removal of catalyst and solvent has to be done with exclusion of air, since otherwise discolourisation takes place. In this manner 2-(2-acetyl)hydrazino-3-amino-pyridine (XXXIX) was obtained in good yield.

Purification of the compound was difficult. Solutions of it coloured on exposure to air. We therefore converted a small amount of the amino derivative in the 2-(2-acetyl)hydrazino-3-acetamidopyridine (XL). This showed the correct analysis.

We also prepared in an analogous way as described for the hydrazinoderivatives: 2-(1-methyl)-hydrazino-3-nitropyridine (XLI), 2-(1-methyl-2-acetyl)hydrazino-3-nitropyridine (XLIII), 2-(1-methyl-2-acetyl)hydrazino-3-aminopyridine (XLIV) and 2-(1-methyl-2-acetyl)hydrazino-3-acetamidopyridine (XLVI).

The position of the methylgroup was ascertained by formation of a hydrazone with acetone. This resulted in 2-(1-methyl-2-isopropylidene)hydrazino-3-nitro-pyridine (XLII).

Ringclosure is apparently not affected during hydrogenation. Abramovitch and Schofield (J.Chem.Soc. (1955) 2326-36) and Temple and coworkers (J.Org.Chem. 28 (1963) 923-7) synthesised respectively benzo-as-triazines and pyrimido-as-triazines. As starting material they used phenyl or pyrimidyl derivatives with an acylated hydrazino-group adjacent to an aminogroup. To affect ringclosure they treated these compounds with hydrochloric acid. The benzo-as-triazines were formed at 95° C and the pyrimido-as-triazines at roomtemperature in hydrochloric acid.

When we hydrogenated 2-(2-acetyl-1-methyl)hydrazino-3-nitropyridine in dilute hydrochloric acid, the right amount of hydrogen was taken up and the solution was deep yellow after hydrogenation.

After removing the solvent under diminished pressure a green solid remained. After recrystallisation from isopropanol, analysis confirmed we had obtained 1,3-dimethyl-1,4-dihydropyrido-(3,2-e)-1,2,4-triazine.hydrochloride (XLV).

The same product could be obtained by solving 2-(2-acetyl-1-methyl)hydrazino-3-aminopyridine in dilute hydrochloric acid.

EXPERIMENTAL PART.

α -aminopropionitrile (I, R=CH₃).

A.N. Baksheev, N.I. Gavrilov: J.Gen.Chem. U.S.S.R. 22 (1952) 2077.

Aminoacetonitrile (I, R=H).

Org.Synthesis Coll. Vol.I, p. 298, 355.

α -carboboxyaminopropionitrile (II, R=CH₃).

Adding to a mixture of 18,5 gr. α -aminopropionitrile and 35 ml dry pyridine, 45 g. carboboxychloride under cooling, gives a dark brown liquid. This reaction mixture is poured out in water and is 3 times extracted with ether. The ether layer is washed with water, acid and again with water until base free. The ether layer is dried on CaCl₂ and evaporated to dryness. The solid residue is repeatedly recrystallised by dissolving in ethanol and precipitation by adding of water.

Yield is 25 g. (40%) m.p. 57.5 - 58.5° C.

Found : C 64.57; H 6.00; N 13.80

Calc. for C₁₁H₁₂N₂O₂ (204): C 64.71; H 5.88; N 13.73%

Carboboxyaminoacetonitrile (II, R=H).

M. Lengelberg, Chem.Ber. 89 (1956) 1185-95.

Carboboxyaminoacetiminoethylether.hydrochloride (III, R=H).

M. Lengelberg, Chem.Ber. 89 (1956), 1185-95.

2,5-dimethyl-3,6-dicarboboxyaminomethyl-2,5-dihydro-1,2,4,5-tetrazine (V).

11 g. (III, R=H) are dissolved in 60 ml absolute alcohol, containing 4.4 g. methylhydrazine. After some time standing at room temperature a white solid separates, which appears to be ammonium chloride. After two days standing a yellow crystalline material separates. This solid is collected and recrystallised from alcohol. Yield 1.9 g. (21%) m.p. 158-9° C.

Found : C 60.38; H 6.05; N 19.05;
60.26; 5.99; 18.85.
Calc. for $C_{22}H_{26}N_6O_4$ (483): C 60.27; H 5.94; N 19.18%

Carbobenzoxyminoacetamidrazone.hydrochloride (IV, R=H).

In analogy to W. Oberhummer, Sitzungsber. 142 (1933), 495-510, 20 g. (III, R=H) is added in 5 minutes to a solution of 3.4 g. methylhydrazine in 200 ml. absolute alcohol at a temperature of -10° C., which is vigorously stirred. When the iminoether has been added, stirring is continued for an hour. The temperature is allowed to rise to 0° C. The solid is collected by suction and recrystallised from absolute alcohol.

Yield 5.0 g. (25%) m.p. $177-8.5^{\circ}$ C.

Found : C 48.73; H 6.17; N 20.47.
Calc. for $C_{11}H_{17}N_4O_2Cl$ (272.5): C 48.44; H 6.24; N 20.55%

Aminoacetamidrazon, dihydrobromide (VI, R=H).

In analogy to M. Lengelberg, Chem.Ber. 89 (1956), 1185-95.

To 1.05 g. (IV, R=H) was added 10 ml glacial acetic acid and 10 ml. of a 30% solution of hydrobromic acid in glacial acetic acid. After a short time a white solid separates, ether is added and the solid collected by suction in quantitative yield. Since it is impossible to purify, we prepared a picrate, m.p. $183-5^{\circ}$ C.

Found : C 31.44; H 3.25; N 24.11
Calc. for $C_3H_{10}N_4 \cdot 2 HOC_6H_2(NO_2)_3 \cdot H_2O$ (578):
: C 31.14; H 3.11; N 24.22%

Carbobenzoxyminoacetiminocyclohexylether.hydrochloride (VII).

M. Lengelberg, Chem.Ber. 89 (1956), 1185-95.

3-methyl-5-carbobenzoxyminomethyl-1,2,4-triazole (VIII).

0.4 g. NaOH in 10 ml methanol was added to a solution of 3.26 g. (VII) in 10 ml methanol. Sodiumchloride is filtered off and the filtrate is added to a solution of 0.75 g. acetylhydrazide in 5 ml. methanol. This solution is refluxed during one hour and concentrated in vacuum. The oily residue was crystallised by

addition of ether. The solid was collected and recrystallised from alcohol. Yield 640 mg. (25%) m.p. 162-3° C.

Found : C 54.48; H 6.17; N 21.13

Calc. for $C_{12}H_{14}N_4O_2 \cdot H_2O$ (264): C 54.44; H 6.06; N 21.21%

3-mercapto-5-hydroxy-1,2,4-triazine-6-carboxylic acid (IX).

R.D. Barlow et al., J.Am.Chem.Soc. 78 (1956) 1258.

3-methylmercapto-5-hydroxy-1,2,4-triazine-6-carboxylic acid (X).

Ibid.

3,5-dihydroxy-1,2,4-triazine-6-carboxylic acid (XI).

Ibid.

3,5-dihydroxy-6-carboethoxy-1,2,4-triazine (XII).

16 g. (II) is refluxed during 5 hours with 200 ml. absolute alcohol and 7.5 ml. concentrated sulphuric acid. After 10 minutes a clear solution is formed. The reaction mixture is concentrated to 50 ml; the solid is collected by suction and washed with alcohol. Recrystallisation from 250 ml ethylacetate.

Yield 13 g. (70%) m.p. 180.5 - 1.5° C.

Found : C 38.62; H 3.78; N 23.27

Calc. for $C_6H_7N_3O_4$ (185): C 38.92; H 3.87; N 22.70%

3,5-dihydroxy-1,2,4-triazine-6-carbohydrazide (XIII).

9.75 g. (XII) is dissolved in 200 ml absolute alcohol; 10 ml. hydrazine hydrate are added and the reaction mixture is refluxed during two hours. After cooling the hydrazine salt of the compound is obtained in a quantitative yield, m.p. 300° C.

Found : C 24.34; H 4.56; N 46.87

Calc. for $C_4H_5N_5O_3 \cdot N_2H_4$ (203): C 23.65; H 4.43; N 48.20%

3,5-dihydroxy-1,2,4-triazine-6-carboxyazide (XIV).

a. In a solution of 1 g. $NaNO_2$ in 2 ml water, 125 mg. (XIII) are

suspended. At a temperature below 5°C , 3 ml. concentrated hydrochloric acid is added under stirring. After forming a clear solution a solid is separated. After 10 minutes the solid is collected, washed with water and dried. Yield 60%, m.p. 150°C (explosion).

b. 5 g. hydrazide are suspended in 200 ml. absolute alcohol; 20 ml. isoamylnitrite are added. Under cooling and stirring 12.5 ml. concentrated hydrochloric acid is added dropwise. After forming a clear solution, the azide crystallises. After a further 30 minutes, the solid is collected and washed with alcohol. Yield 60-70%.

Literature: a. V.J. Schut et al. Recueil 80 (1961) 391.

b. H. Jensen et al. J.Am.Chem.Soc. 48 (1926) 1988.

3,5-dihydroxy-6-carboethoxyamino-1,2,4-triazine (XV).

25 mg. azide (XIV) is heated in 2 ml. absolute alcohol in about 30 minutes to 80°C . At 60°C gas development is observed. After 45 minutes refluxing and subsequent cooling, 21 mg. (70%) of the compound is obtained.

Found : C 36.15; H 4.04; N 28.09

Calc. for $\text{C}_6\text{H}_8\text{N}_4\text{O}_4$ (200): C 36.00; H 4.00; N 28.00%.

3,5-dihydroxy-6-carbobenzoxamino-1,2,4-triazine (XVI).

1,5 g. azide (XIV) is suspended in a solution of 2.5 ml. benzylalcohol in 50 ml. dry toluene. In 10 minutes this reaction mixture is brought to 100°C and held for 30 minutes at this temperature. After a further 90 minutes refluxing the mixture is cooled and filtrated. The solid is washed with toluene. Yield 2.0 g. (92%), m.p. $256-9^{\circ}\text{C}$.

Recrystallisation from 500 ml. alcohol.

Found : C 50.59; H 4.03; N 21.32

Calc. for $\text{C}_{11}\text{H}_{10}\text{N}_4\text{O}_4$ (262): C 50.38; H 3.82; N 21.37%.

3,5-dihydroxy-6-amino-1,2,4-triazine (XVII).

a. 750 mg. benzylurethane (XVI) is refluxed during 30 minutes

with 8 ml. of a 30% solution of hydrobromic acid in glacial acetic acid. After cooling the solid is collected in quantitative yield.

b. 262 mg. benzyl urethane is hydrogenated in 60 ml. glacial acetic acid with Pd/C 10% catalyst. After 45 minutes at room-temperature and atmospheric pressure, reduction is complete. Removal of catalyst and solvent gives the compound in a quantitative yield.

c. 4 g. azide (XIV) is gently heated in 50% acetic acid. At 60°C gas development occurs, which becomes vigorous at 70°C. The solid is collected after a further 20 minutes at 80°C and 10 minutes at 100°C. Recrystallisation from 275 ml. water. Yield 2.6 g. (92%), m.p. 300°C.

d. 50 mg. azide in 20 ml water is gently heated to 100°C. After 15 minutes boiling, the reaction mixture is cooled and the solid collected. Yield 130 mg.

Literature: J. Bäumler et al., *Helv.Chim.Acta* 34 (1951) 496.

e. P.K. Chan, *J.Org.Chem.* 26 (1961) 1118-20 synthesised the same product via the intermediates 3,5-dihydroxy-1,2,4-triazine (XVIII) and 3,5-dihydroxy-6-bromo-1,2,4-triazine (XIX).

Benzoyl pyruvic methylester (XX).

H. Freri, *Gazz.Chim.Italia* 68 (1938) 612-18.

3-phenyl-4-nitroso-5-carbomethoxypyrazole (XXI).

R. Fusco, S. Rossi, *Tetrahedron* 2 (1958) 209.

3-phenyl-4-amino-5-carbomethoxypyrazole (XXII).

Ibid.

1-methyl-4-phenyl-2,7-dioxo-1,2,3,7-tetrahydropyrazolo-(3,4-d)-pyrimidine (XXIII).

A mixture of 4 g. (XXII), 4 g. methylisocyanate, 0.6 ml. triethylamine and 30 ml. benzene is heated during 10 hours at

100° C in a Carius tube. The reaction mixture is evaporated to dryness and the residue is boiled during 10 minutes with 25 ml. 2N KOH. The clear solution is three times extracted with 25 ml. portions ether. The water layer is brought to pH 6 with acetic acid and the formed solid is collected and washed with water. Recrystallisation from 50% acetic acid or methanol. Yield 2 g. (44%), m.p. 350-370° C after sublimation at 250-300° C. Found : C 59.44; H 4.13; N 23.11
Calc. for $C_{12}H_{10}N_4O_2$ (218): C 59.50; H 4.13; N 23.14%.

2-amino-3-nitropyridine (XXIV).

L.N. Pino, W.S. Zehrung, J. Am. Chem. Soc. 77 (1955) 3154.

2-methylamino-3-nitropyridine (XXV).

A.E. Tschitschibabin, A.V. Kirssanow, Ber. 61 (1928) 1228.

2-methylnitramino-3-nitropyridine (XXVI).

Ibid. p. 1232.

2-nitrosomethylamino-3-nitropyridine (XXVII).

Ibid. p. 1230.

2-amino-5-nitropyridine (XXVIII).

See 2-amino-3-nitropyridine.

2-hydroxy-5-nitropyridine (XXIX).

M.A. Phillips, J. Chem. Soc. (1941) 9.

2-hydroxy-3,5-dinitropyridine (XXX).

E. Plazek, Recueil 72 (1953) 569-75.

2-chloro-3,5-dinitropyridine (XXXI).

Ibid.

2-(1-methyl)hydrazino-3,5-dinitropyridine (XXXII).

3.4 g. 2-chloro-3,5-dinitropyridine (XXXI) is dissolved in 40 ml. cold methanol and added dropwise under stirring to a solution of 3.1 g. methylhydrazine in 20 ml. methanol. After standing overnight the solid is collected by suction and washed with methanol. Recrystallisation from 60 ml. ethanol. Yield 2.6 g. (73%), m.p. 147.5 - 8.5° C.

Found : C 34.00; H 3.33; N 32.68.

Calc. for $C_6H_7N_5O_4$ (213): C 33.80; H 3.29; N 32.86%.

2-(1-methyl-2-isopropylidene)hydrazino-3,5-dinitropyridine
XXXIII.

100 mg. methylhydrazine compound (XXXII) is dissolved in 1 ml. acetone. One drop of concentrated hydrochloric acid is added. Diluting with water gives a yellow crystalline compound. Recrystallisation by dissolving in acetone and adding water. Yield 80 mg., m.p. 114.5 - 5.5° C.

Found : C 42.78; H 4.44; N 27.47.

Calc. for $C_9H_{11}N_5O_4$ (253): C 42.69; H 4.35; N 27.67%.

2-(1-methyl-2-acetyl)hydrazino-3,5-dinitropyridine (XXXIV).

1 g. methylhydrazino compound (XXXII) is dissolved in 20 ml. acetic anhydride. After standing overnight at roomtemperature, excess acetic anhydride is removed under reduced pressure. Recrystallisation from 20 ml. 50% alcohol.

Yield 1.15 g. (95%), m.p. 177 - 8.5° C.

Found : C 37.54; H 3.56; N 27.33

Calc. for $C_8H_9N_5O_5$ (255): C 37.65; H 3.53; N 27.45%.

2-(1-methyl-2-acetyl)hydrazino-3,5-diacetamidopyridine (XXXVI).

291 mg. of the dinitro derivative (XXXIV) was hydrogenated with 98 mg. Pd/C 10% in 60 ml. alcohol. Reduction is complete in two hours. After removing catalyst and solvent with exclusion of oxygen, the residue was taken up in acetic anhydride. To dissolve the residue it was heated to 70-80° C. After standing overnight the solid was collected and washed with 2 ml. acetic anhydride.

Recrystallisation from ethylacetate gave 190 mg., m.p. $242.5-3^{\circ}$ C.
Found : C 51.78; H 6.06; N 25.09
Calc. for $C_{12}H_{17}N_5O_3$ (279): C 51.61; H 6.09; N 25.09%.

2-hydrazino-3-nitropyridine (XXXVII).

Dissolve 5 g. 2-chloro-3-nitropyridine in 50 ml. hot alcohol. This solution is added slowly to a solution of 5 ml. hydrazine-hydrate in 10 ml. alcohol. Recrystallisation from 130 ml. 50% alcohol. Yield 4.42 g. (90%), m.p. $167.5 - 9^{\circ}$ C.

Found : C 39.00; H 3.97; N 36.52
Calc. for $C_5H_6N_4O_2$ (154): C 38.96; H 3.90; N 36.36%.

Sym.,-di-2,2'-(3-nitropyridyl)hydrazine (XXXVIIa).

1100 mg. 2-chloro-3-nitropyridine is dissolved in 30 ml. warm butanol. 0.5 ml. hydrazine-hydrate is added and the solution is refluxed during three hours. The reaction mixture is hot filtered. The red crystalline residue is recrystallised from glacial acetic acid (60 ml). Yield 320 mg. (33%), m.p. $275-80^{\circ}$ C. (dec.).

2-(2-acetyl)hydrazino-3-nitropyridine (XXXVIII).

Dissolve 2.5 g. hydrazino compound (XXXVII) in 50 ml acetic anhydride. After standing overnight at roomtemperature excess anhydride is removed under reduced pressure. Recrystallisation by continuous extraction with petroleum ether b.p. $60-80^{\circ}$ C.
Yield 2.95 g. (92%), m.p. $151-5 - 3^{\circ}$ C.

Found : C 42.64; H 4.20; N 28.37
Calc. for $C_7H_8N_4O_3$ (196): C 42.86; H 4.08; N 28.75%.

2-(2-acetyl)hydrazino-3-aminopyridine (XXXIX).

499 mg. of the nitro compound (XXXVIII) are hydrogenated at room-temperature and atmospheric pressure with 93 mg. Pd/C (10%) in 60 ml. alcohol. Hydrogenation is complete in two hours. After removing catalyst and evaporating alcohol under nitrogen, the residue crystallises. Yield 311 mg. (70%), m.p. $150 - 2^{\circ}$ C. Due to its susceptibility to oxygen it is impossible to purify. We acylated it therefore to (XL).

Found: C 43.69; H 3.06; N 30.28. Calc. for $C_{10}H_8N_6O_4$ (276):
C 43.48; H 2.90; N 30.43%

3-acetamido-2-(2-acetyl)hydrazinopyridine (XL).

146 mg. of the crude amino derivative (XXXIX) is dissolved in 5 ml. acetic anhydride. After some time standing, the solid is collected and recrystallised from a mixture of ethylacetate and petroleum ether b.p. 80-110° C. Yield 61 mg. (33%), m.p. 178-80°C.
Found : C 52.26; H 5.74; N 26.60
Calc. for $C_9H_{12}N_4O_2$ (208): C 51.92; H 5.77; N 26.92%.

2-(1-methyl)hydrazino-3-nitropyridine (XLI).

To a solution of 6.25 g. methylhydrazine in 90 ml. water is added 10 g. 2-chloro-3-nitropyridine. The reaction mixture is refluxed untill all pyridine has dissolved. Cooling gives 9.7 g. (90%) of a yellow solid, which can be recrystallised from a mixture of n-pentane-ether 1:1, m.p. 59-60.5° C.
Found : C 42.96; H 4.79; N 32.68
Calc. for $C_6H_8N_4O_2$ (168): C 42.86; H 4.76; N 33.33%

2-(1-methyl-2-isopropylidene)hydrazino-3-nitropyridine (XLII).

1 g. hydrazino compound (XLI) is dissolved in 4 ml. acetone. Two drops concentrated hydrochloric acid are added and the solution is diluted with water. The yellow solid is collected and recrystallised from acetone-water. Yield 800 mg. (64%), m.p. 95-6° C.
Found : C 52.22; H 5.76; N 26.63
Calc. for $C_9H_{12}N_4O_2$ (208): C 51.92; H 5.77; N 26.92%.

2-(1-methyl-2-acetyl)hydrazino-3-nitropyridine (XLIII).

7 g. hydrazino derivative (XLI) is dissolved in 30 ml. acetic anhydride. After standing overnight and removing excess anhydride under reduced pressure the solid is collected. Recrystallisation from ethylacetate-petroleum ether. Yield 7 g. (80%), m.p. 151-2°C.
Found : C 45.99; H 4.87; N 26.46
Calc. for $C_8H_{10}N_4O_3$ (208): C 45.71; H 4.76; N 26.67%.

2-(1-methyl-2-acetyl)hydrazino-3-aminopyridine (XLIV).

843 mg. of the nitro derivative (XLI) is hydrogenated at room-temperature and atmospheric pressure in 60 ml. alcohol with 44 mg. Pd/C (10%). Reduction is complete in two hours. The catalyst is filtered and the alcohol is removed under nitrogen and diminished pressure. The residue is recrystallised from ethylacetate-petroleum ether 1:3. Yield 478 mg. (66%), m.p. 125-6.5° C.

Found : C 53.46; H 6.74; N 31.23

Calc. for $C_8H_{12}N_4O$ (180): C 53.33; H 6.67; N 31.11%

2-(1-methyl-2-acetyl)hydrazino-3-acetamidopyridine (XLVI).

The hydrogenation product of 220 mg. nitro derivative (XLIII) is taken up in acetic anhydride. Removing excess anhydride under reduced pressure and recrystallisation of the residue from ethylacetate/petroleum ether gives 155 mg. (67%), m.p. 164.5 - 6° C.

Found : C 54.18; H 6.34; N 25.35.

Calc. for $C_{10}H_{14}N_4O_2$ (222): C 54.05; H 6.31; N 25.23%

1,3-dimethyl-1,4-dihydro-pyrido(3,2-e)-1,2,4-triazine.hydrochloride. (XLV).

a. 621 mg. nitro derivative (XLIII) is hydrogenated at room temperature and atmospheric pressure with 66 mg. Pd/C (10%) in 40 ml. 2N HCl. Reduction is complete in two hours. A deep yellow solution remains after removing the catalyst. After evaporating the solvent, the residue was recrystallised from iso-propanol. Yield 390 mg. (65%), m.p. 215 - 20° C (dec.).

Found : C 48.50; H 5.63; N 28.41; Cl 18.06

Calc. for $C_8H_{10}N_4.HCl$ (198.5): C 48.36; H 5.54; N 28.21; Cl 17.89%

b. 140 mg. amino derivative (XLIV) was dissolved in 10 ml. 2N HCl. The solution was immediately evaporated to dryness. The residue was recrystallised, after drying over NaOH, from 30 ml. isopropanol. Yield 120 mg. of the same product.

2-(1-methyl-2-acetyl)hydrazino-3-aminopyridine (XLIV).

843 mg. of the nitro derivative (XLI) is hydrogenated at room-temperature and atmospheric pressure in 60 ml. alcohol with 44 mg. Pd/C (10%). Reduction is complete in two hours. The catalyst is filtered and the alcohol is removed under nitrogen and diminished pressure. The residue is recrystallised from ethylacetate-petroleum ether 1:3. Yield 478 mg. (66%), m.p. 125-6.5° C.

Found : C 53.46; H 6.74; N 31.23

Calc. for $C_8H_{12}N_4O$ (180): C 53.33; H 6.67; N 31.11%

2-(1-methyl-2-acetyl)hydrazino-3-acetamidopyridine (XLVI).

The hydrogenation product of 220 mg. nitro derivative (XLIII) is taken up in acetic anhydride. Removing excess anhydride under reduced pressure and recrystallisation of the residue from ethylacetate/petroleum ether gives 155 mg. (67%), m.p. 164.5 - 6° C.

Found : C 54.18; H 6.34; N 25.35.

Calc. for $C_{10}H_{14}N_4O_2$ (222): C 54.05; H 6.31; N 25.23%

1,3-dimethyl-1,4-dihydro-pyrido(3,2-e)-1,2,4-triazine, hydrochloride. (XLV).

a. 621 mg. nitro derivative (XLIII) is hydrogenated at room temperature and atmospheric pressure with 66 mg. Pd/C (10%) in 40 ml. 2N HCl. Reduction is complete in two hours. A deep yellow solution remains after removing the catalyst. After evaporating the solvent, the residue was recrystallised from iso-propanol. Yield 390 mg. (65%), m.p. 215 - 20° C (dec.).

Found : C 48.50; H 5.63; N 28.41; Cl 18.06

Calc. for $C_8H_{10}N_4.HCl$ (198.5): C 48.36; H 5.54; N 28.21; Cl 17.88%

b. 140 mg. amino derivative (XLIV) was dissolved in 10 ml. 2N HCl. The solution was immediately evaporated to dryness. The residue was recrystallised, after drying over NaOH, from 30 ml. isopropanol. Yield 120 mg. of the same product.